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Preformulation of cysteamine gels for treatment of the ophthalmic complications in cystinosis

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ABSTRACT

Nephropathic cystinosis is a rare autosomal recessive disease characterised by raised lysosomal levels of cystine in the cells of all organs. It is treated by regular administration of the aminothiol, cysteamine. Corneal crystal deposition is one of the most troublesome complications affecting patients and requires the hourly administration of cysteamine eye drops. In an attempt to reduce this frequency and improve the treatment, the preformulation and evaluation of cysteamine containing gels is reported. Suitability for ophthalmic delivery was determined by analysis of rheology, bioadhesion, dissolution and stability. The results demonstrated that three polymers were suitable for ophthalmic delivery of cysteamine; namely sodium hyaluronate, hydroxyethyl cellulose and carbomer 934. Sodium hyaluronate displayed optimum performance in the preformulation tests, being pseudoplastic (reduction in apparent viscosity under increasing shear rate), bioadhesive, releasing cysteamine over 40 min and displaying stability over time. In conclusion these results offer the possibility to formulate cysteamine in an ocular applicable gel formulation.

1. Introduction

Nephropathic Cystinosis is a rare genetic disease, characterised by extremely high lysosomal levels of cystine, the oxidized dimer form of the amino acid cysteine and manifested by a general failure to thrive (Buchan et al., 2012). The accumulation of cystine as crystals in most tissues leads to the progressive impairment and dysfunction of multiple organs, such as the pancreas, eye, brain and thyroid (Syres et al., 2009). Without treatment, this autosomal recessive disease can result in multi-organ failure and death before the onset of puberty (Gahl, 2009). The main treatment for the disorder remains the administration of the aminothiol, cysteamine (Fig. 1) (as the bitartrate salt, Cystagon®) (Thoene et al., 1995). Cysteamine therapy produces rapid depletion of cystine from leukocytes, with minor side effects (Schneider et al., 1976; Schneider, 2004).

Corneal crystal deposition is one of the most troublesome complications affecting patients with cystinosis and persists even as their prognosis improves and life expectancy increases. Photophobia and, ultimately, blepharospasm affect the quality of life to such an extent that the slightest glimmer of sunlight can be debilitating. In addition, crystal accumulation over a period of years can cause the formation of corneal scars, keratitis and cataracts, as well as band keratopathies (Gahl and Kuehl, 2000). The oral form of Cystagon has no effect on depleting corneal crystals due to poor drug availability stemming from the absence of vasculature in the cornea (Gahl et al., 1987; Gahl and Kuehl, 2000), thus cysteamine must also be administered topically in the form of eye drops.

Compliance with the use of eye drops is a major issue however, as in order to achieve the maximum benefit in their current formulation these drops must be routinely administered every hour while awake (Gahl and Kuehl, 2000; Gahl et al., 2007). This is due to a low bioavailability commonly reported for topical eye treatments, with tissue contact time varying from 1 to 2 min (Gangrade et al., 1996; Jansook et al., 2010; Le Bourlais et al., 1998) to 5 min (McKenzie and Kay, 2015; Robinson, 1989; Shell, 1984; Urtti and Salminen, 1993). This short ophthalmic residence time is due to a multitude of protective mechanisms such as blinking and high tear fluid production and turnover (Ahmed and Patton, 1987; Kaur and Kanwar, 2002; Saettone and Salminen, 1995; Morrison and Khutoryanskiy, 2014a,b; Morrison et al., 2013). The current drops, which contain 0.55% cysteamine hydrochloride in saline also cause frequent stinging and redness upon application (Gahl, 2009).

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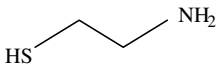

Fig. 1. Cysteamine.

By formulating cysteamine as a bioadhesive ophthalmic gel with controlled drug release, it is hypothesised that administration frequency may be reduced, perhaps allowing once or twice-daily dosing instead of the current hourly requirement. These formulation changes may reduce the burden of treatment and improve compliance, producing long-term prevention of ophthalmic morbidity, such as blindness. Hydrogels which are pseudoplastic, transparent and bioadhesive are highly desirable for topical ophthalmic application, and have the potential for less frequent application and improved patient compliance.

2. Materials and methods

Cysteamine hydrochloride (CH), hydroxyethyl cellulose (HEC), hydroxypropylmethyl cellulose (HPMC), xanthan gum (XG), potassium chloride, sodium chloride, sodium carbonate, calcium carbonate and magnesium chloride were purchased from Sigma, UK. Polyvinylpyrrolidone (PVP) was purchased from Fluka. Ellman's Reagent, 5,5′-dithiobis(2-nitrobenzoate) (DTNB) was purchased from Molekula (Gillingham, UK). Tris buffer (1 M, pH7.4) was bought from Fisher. Carbomer 934 (C934) was purchased from Universal Biologicals, UK. Carbomer 974 (C974) was purchased from Surfachem, UK. Sodium hyaluronate (HA) was purchased from Aromantic (Moray, UK). Benzalkonium chloride (BZK) was purchased from Aldrich. Tubing membrane (12–14,000 kDa) was purchased from Visking, UK. All other chemicals were of pharmaceutical grade.

As part of initial pre-formulation work, potential gel carriers were screened for suitability as ophthalmic vehicles. A literature review was undertaken, investigating the suitability of polymers available for ophthalmic use. Inclusive parameters were non-toxicity in the eye, pseudoplastic rheology, bioadhesive nature, good optical clarity, stability and compatibility with cysteamine. Eight gel carriers met these criteria. The eight gels were subjected to unmedicated pre-formulation testing of rheology and optical clarity. The polymers were dissolved in water for injection (WFI) and neutralised to pH 7.4 with sodium hydroxide if required, and allowed to fully hydrate at 4° C for 24 h before testing. The concentrations used corresponded to those used commercially (Table 2). This maintained the apparent viscosity of the gels within the limits tolerated by the eye. Gels which performed satisfactorily were loaded with 0.55%w/w CH and subjected to further testing: dissolution, bioadhesion, toxicity, stability and surface tension. Unmedicated gels were used as controls. Simulated Lachrymal Fluid (SLF) at pH 7.4 was used to mimic tear fluid during tests. It was also used in the production of the ophthalmic gels to provide additional buffering capacity. The following salts were weighed out and stirred in a 1 l volumetric flask: potassium chloride 0.179% w/v, sodium chloride 0.631% w/v, sodium carbonate 0.218% w/v, calcium carbonate 0.004% w/v and magnesium chloride 0.005% w/v. Hydrochloric acid (0.1 M) was used to adjust the pH to 7.4. Sorensen's Modified Phosphate Buffer (SMPB) was also added to the gels to stabilise the gels at pH7.4. It is one of the most common ophthalmic buffers used. Briefly, a solution of 0.2 M monosodium phosphate and disodium phosphate was made in WFI.

2.1. Rheology

The rheological properties of the gel were studied using an Advanced Rheometer AR1000 from TA Instruments (Delaware, USA). A 60 mm, 2° angle cone geometry was used, with a truncation value of 65 μm. All measurements were made at 34 °C, the temperature at the corneal surface. Continuous shear measurements were made initially, using a linear mode and a continuous ramp of 0–600 s^{-1}, and 600–0 s^{-1} over 20 min, to establish flow types such as Newtonian or pseudoplastic.

Oscillatory measurements were performed on the gels to characterise the linear visco-elastic behaviour and relate the rheological parameters to molecular structure. A linear mode was used with a frequency of 1–10 Hz, and 20 sample points. The controlled variable was percentage strain. The sample volume was approximately 1.5 ml. All tests were performed in triplicate.

2.2. Optical transmission

The optical transmission of each gel was measured using a Cecil CE 3021 Spectrometer (Cambridge, England). The transmission was measured as a ratio of the amount of light unabsorbed by the gel to the total amount of light the gel was exposed to, expressed as a percentage. A wavelength of 480 nm was used, the middle of human light wavelength perception. The gels were measured with 1 cm, 5 mm and 2 mm path lengths; although *in situ* they would be less than a millimetre thick. The gels were referenced to deionised water at room temperature, which was taken as 100% transmission. A figure greater than 90% was classed as transparent, between 10 and 90% as translucent, and less than 10% as opaque (Buchan et al., 2010). Each test was performed in triplicate.

2.3. Evaluation of cysteamine release via dissolution

A 100 ml round-bottomed flask was held in a water bath, heated to 34 °C. To the flask 50 ml aqueous solution of SLF and Tris buffer (90:10) was added, and an equimolar quantity (to the concentration of cysteamine in the gel) of Ellman's reagent was added, and this solution was stirred magnetically using an IKA RET basic hotplate stirrer (Staufen, Germany). A dialysis membrane (12–14,000 kDa) containing 7 ml of the gel and, tied in a rod shape (length 2.23 cm; radius 1 cm, average of 3 measurements) to exclude air bubbles, was added to the flask at time zero. The quantity of gel used was chosen to avoid reaching the limit of detection of the UV spectrometer. The medium was sampled every 2 min for the first ten minutes, every 5 min for an hour, and every 15 min after the first hour. Samples were analysed at 440 nm, the λ_{max} for DTNB. Dissolution tests were performed in triplicate.

2.4. Bioadhesion

Bovine corneal tissue, which is reported as being similar in structure to the human cornea, was used as a control (Loch et al., 2012). A Texture Analyser (Stable Micro Systems, Surrey, UK) was used to measure the force required to remove the gel from an area of bovine cornea (Thirawong et al., 2007). Fresh bovine eyes were collected immediately after slaughter, and washed with deionised water. The whole cornea was then excised and washed in SLF at room temperature. Prior to testing, the corneas were placed on a tissue to remove excess fluid. Cyanoacrylate glue was then used to attach a cornea to a 2 cm^2 stainless steel plate. Care was taken not to allow the glue to come into contact with the upper surface of the tissue. Immediately after this, the steel plates were attached (in pairs) to the Texture Analyser, one positioned directly above the other. Each gel sample was placed between the cornea samples and held together for 60 s; the force required to separate the plates was then measured (contact force of 0.05 N, contact time 60 s, probe speed 0.5 mm/s). The force was plotted against distance; the area under the curve (AUC) being equal to the work of adhesion (Wad) (Thirawong et al., 2007; Varum et al., 2010). Each individual test